PATENT
395/35

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Gerard M. Housey
Serial No.	:	09/510,562
Filing Date	:	February 22, 2000
For	:	METHOD FOR SCREENING FOR PROTEIN INHIBITORS AND ACTIVATORS
Examiner	:	Guzo, D.
Art Unit	:	1636

Assistant Commissioner for Patents
Washington, D.C. 20231

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By: Lawrence P. Casson (Reg. No. 46,606)

AMENDMENT

SIR:

The Applicant acknowledges the Examiner's courtesy in meeting with the Applicant and his attorney, Mr. Steven J. Lee, on December 20, 2001, to discuss issues common to the above-identified patent application and copending Ser. No. 09/510,554. This is in response to the Office Action mailed February 4, 2002 in the above-identified patent application and is accompanied by a petition for a three month extension of time extending the time for response up to and including August 5, 2002. Claims 33, 34, 36, 37, 43-50, 59-65 and 71-78 were examined. Favorable reconsideration and allowance of the application is respectfully requested.

Rejection Under 35 U.S.C. § 102(b)

Claims 33-34, 36, 43-44, 46-47, 49, 63-64, 71-72, 74-75, and 77 have been rejected under 35 U.S.C. § 102(b) as anticipated by Drebin et al. ("Drebin").

In order for a cited reference to anticipate under 35 U.S.C. § 102(b), that reference must disclose each and every limitation of the instant claim. The instant claims are directed to inhibition or activation of an enzyme in a cell using a chemical agent identified to be an inhibitor or an activator of the enzyme. By inhibitor or activator, what is meant is an inhibitor or activator of the activity of the enzyme. According to the invention, such inhibitors and activators can be identified by their effect on a responsive phenotypic characteristic evoked by production of the protein in a cell. The change in the responsive phenotypic characteristic is *other than* a change in the level of the protein-of-interest (POI).

Drebin does not anticipate the claimed invention. First, the claims recite chemical agents whereas Drebin discloses an antibody. It would be clear to one of skill in the art, based on the disclosure and Example 1 in particular, that the claimed invention relates to inhibitors and activators of enzymatic activity, and not antibodies. Antibodies are generally thought of as binding proteins and not inhibitors or activators in the usual sense of these words. There are also commonly used distinctions between chemical agents and biological agents that are generally accepted. For example, in reviewing New Drug Applications, the Food and Drug Administration (FDA) generally distinguishes between chemical agents, which are assigned to the Center for Drug Evaluation and Research (CDER) and biological products, which include protein and antibody based drugs and which are assigned to the Center for Biologics Evaluation and Research (CBER). See Exhibit A.1, *Intercenter Agreement Between the Center for Drug Evaluation and Research and the Center for Biologics Evaluation and Research*, Sections III(A.) and (B.). The agreement outlines the responsibilities of the two centers which trace their history through the formation of the National Center for Drugs and Biologics (early 1980's) and its subsequent split in October,

1987. See Exhibit A.2, *A Brief History of the Center for Drug Evaluation and Research*, pages 7-9.

The scientific literature prior to and at the time the application was filed further supports that "chemical agents" is a term of art that does not include antibodies. Cahn, J.Y. et al., *Autologous bone marrow transplantation (ABMT) for acute leukaemia in complete remission: a pilot study of 33 cases*, Brit. J. Haematol. 63:457-470 (1986) (Exhibit B) describes purging of marrow by a chemical agent or by monoclonal antibodies and complement. (See, Summary, and Methods (b) *Ex-vivo treatment* (i) *Exposure to a chemical agent* and (ii) *Monoclonal antibodies and complement mediated cytolysis*. For an example describing the use of "chemical agents" to induce differentiation, and referring to monoclonal antibodies for monitoring the presence of differentiation antigens, see Perussia, B. et al., *Terminal differentiation surface antigens of myelomonocytic cells are expressed in human promyelocytic leukemia cells (HL60) treated with chemical inducers*, Blood 58:836-43 (1981) (Exhibit C; see, e.g., Abstract and Table 4; chemical agents used to induce differentiation are dimethyl sulfoxide (DMSO), retinoic acid, 12-O-tetradecanoyl-phorbol-13-acetate (TPA)).

More importantly, Applicant's claimed invention can be distinguished from Drebin or other such prior art even if antibodies are construed as within the scope of chemical agents. Significant differences exist between the disclosure of Drebin and the instant claimed invention. For example, Drebin does not teach cells that exhibit a phenotypic characteristic that is responsive to the inhibition or activation of the target enzyme (*i.e.*, the *neu*-oncogene product; p185), nor does Drebin teach the use of cells that exhibit a responsive phenotypic characteristic other than the level of the target enzyme.

Drebin teaches only that "addition of [a monoclonal antibody specific for p185] to cultured [cells expressing p185] causes rapid down-modulation of cell surface p185 expression. This modulation persists as long as cells are cultured in the presence of antibody, and is reversed when antibody is eliminated from the culture media." (Drebin, p. 696,

Col. 2). Drebin further correctly cites well known literature going back almost 20 years prior to Drebin's manuscript that report similar observations, *i.e.*, that antibodies directed toward cell-surface antigens cause progressive down-modulation (loss) of the protein from the cell surface. In addition, as well known in the art, Drebin recognizes that "divalent IgG molecules are capable of inducing antigenic modulation, whereas their monovalent F(ab) fragments are not." (*ibid.*) In this regard, Drebin also shows that F(ab) fragments, which are still able to bind to cell surface p185, do not cause its down regulation (Drebin, Fig. 3 and p. 697, Col. 2) and do not revert the transformed phenotype. Thus, Drebin does not disclose any responsive change in a phenotypic characteristic evoked by the production of the protein in the cell, but only a change in the level of the POI. Drebin's work essentially removed the POI from the cell, thereby precluding the ability to practice Applicant's invention.

Statements by the Opposition Division of the European Patent Office with regard to Drebin (designated reference A9) are consistent with Applicant's position. "The Opposition Division notes that such antibody-mediated down-modulation of cell surface p185 does not result in inhibition of a given target protein as defined in the patent." (Exhibit D; Summons to Attend Oral Proceedings Pursuant to Rule 71(1) EPC, April 21, 1999, with Provisional Opinion, page 19). In summary, this three member panel determined that there appeared to be a "fundamental difference between the methods of the patent and the experiments reported in A9." (Exhibit E; Interlocutory decision in Opposition Proceedings (Article 106(3) EPC), April 26, 2000, page 12, para. 22a.) Exhibits D and E have been previously disclosed.

One of ordinary skill in the art would take Drebin as evidence that the effects of the overproduction of an integral membrane protein in a cell can be eliminated by adding an agent that destroys the overproduced protein, thereby returning the phenotype to the unaltered state. In contrast, the claimed inhibitors and activators bring about a change in a responsive phenotypic characteristic by inhibiting or activating the enzyme. Drebin does not

teach or suggest that the disclosed antibodies affect the enzymatic activity of the receptor according to the teachings of Applicant's method. Accordingly, the invention is not anticipated by Drebin and the rejection should be withdrawn.

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 33-34, 36-37, 43-50, 59-65, and 71-78 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter that is not enabled. It is asserted that additional undisclosed experimentation would be necessary to distinguish between chemical agents which directly interact with the POI so as to inhibit or activate the protein or enzyme of interest (POI) and chemical agents which act indirectly to inhibit or activate the POI.

Applicant respectfully disagrees. The disclosure, alone or coupled with information known in the art, enables one skilled in the art to use the claimed invention. As described in detail in the specification, Applicant's pioneering invention is focused on the creation and utilization of cellular assay systems specifically designed to identify substances capable of activating or inhibiting a given POI by interacting with (*i.e.*, binding to) the POI to exert their modulating effects. This is stated, for example, in the Specification and in the original patent (U.S. Patent 4,980,281; "'281 patent") as follows:

> This invention relates to a general screening method for the discovery and identification of both inhibitors and activators of enzymes, receptors, and other proteins. In particular, it is concerned with a method of screening for substances which *specifically* inhibit or activate a particular protein . . .

Specification, p. 1, ll. 12 to 17; '281 patent, col. 1, ll. 7 to 12 (emphasis added).

> In brief, the method which we describe herein involves the generation of a cell line purposefully engineered to detect both stimulatory and inhibitory agents which are *absolutely specific* for any given protein . . .

Specification, p. 3, l. 34 to p. 4, l. 4 ; '281 patent, col. 2, ll. 27 to 32 (emphasis added).

> Thus, this work establishes, for the first time, the fact that stable overproduction of a protein in mammalian cells can

> result in a novel cellular phenotype(s) (in this case anchorage independence) *which can be directly modulated by chemical agents which interact with the protein.*

Specification, p. 39, ll. 21 to 26; '281 patent, col. 18, ll. 30 to 36 (emphasis added).

Assays to detect simple binding of chemical agents to proteins or enzymes were in common use at the time of the invention and remain so today. One skilled in the art would certainly know to use such an assay to test the interaction between (*i.e.*, binding of) a chemical agent and a given POI if so desired. The Examiner's attention is directed to, for example, page 31, line 5 to page 32, line 15 of the specification, where an intact cell assay is used to assess binding of a phorbol ester ([^{3}H]phorbol 12, 13-dibutyrate; PDBU) in cell lines that express differing amounts of the β1 isoform of PKC. Details of PDBU binding are disclosed for cell lines R6-PKC1, R6-PKC3, R6-PKC4, R6-PKC5 and R6-PKC6 in Table 1(a). These and other such binding assays using intact cells or purified proteins are well known in the art. (See, *e.g.,* Horowitz et al., 1981, Proc. Natl. Acad. Sci. USA 78:2315-2319, and other binding assays cited in the specification.)

Although the scope of the claims is broad, the nature of the invention is broad, concerning as it does to a general method for inhibiting or activating any given POI. The instant claims are directed to a method of inhibiting or activating a protein or enzyme in a cell, using a chemical agent identified to be an inhibitor or activator of the protein in a cell by the particularly recited steps. Even if assays for determining direct interaction had not been disclosed, one of skill in the art would know how to perform such assays as is evidenced by the literature cited above and in the specification.

The paper by Hsiao et al. ("Hsiao") has been cited as evidence that by the method of the invention, TPA or teleocidin, for example, would be misidentified as a chemical agent that directly interacts with c-Ha-ras. However, Hsiao has not devised an assay system which can identify a change in a responsive phenotypic characteristic as set forth by the inventor. Where Hsiao relates focus formation in Rat-6 cells to TPA treatment,

there is no determination of c-Ha-ras levels in the transformed cells (e.g., Table 1). Hsiao discloses that cell transformation appears to be related to retention of enhancer sequences adjacent to the c-Ha-ras coding sequence in pT24 DNA and discloses that TPA treated cells had much higher levels of pT24 RNA (p. 1947, Col. 2, lines 9-26). One of ordinary skill in the art would understand this to mean that c-Ha-ras expression increases in response to TPA treatment.

To the contrary, Applicant's claims recite a change in a responsive phenotypic characteristic other than the level of the protein *per se*. Furthermore, the skilled artisan would understand the desirability of using a controlled expression system for c-Ha-ras, *i.e.*, a system in which the observed phenotype does not depend on retention or loss of enhancer sequences, or other factors affecting gene expression. Controlled expression systems and methods of determining amounts of protein and levels of gene expression were well known in the art, but were not utilized by Hsiao. Indeed, since these authors have simply transfected entire cell cultures with the pT24 plasmid and scored for focus formation, such foci contain widely differing levels of p21 ras protein. Thus, there is no specific association between the level of a given target protein and a corresponding responsive change in a phenotypic characteristic evoked by the presence of the protein in the cell.

Accordingly, Applicant asserts that the claims are fully enabled and respectfully requests that the rejection be withdrawn.

Claims 33-34, 36-37, 43-50, 59-65, and 71-78 have been rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not described so as to convey that the inventor had possession of the claimed invention. The Examiner asserts that the disclosed method does not discriminate between chemical agents that directly or indirectly interact with the POI.

Applicant respectfully disagrees with this position. As discussed above, the specification describes, in detail, the creation and utilization of cellular assay systems

specifically designed to identify substances capable of activating or inhibiting a given POI by interacting directly with (*i.e.*, binding to) the POI. For example, the specification discloses that by looking for an increase in a phenotypic change exhibited by a cell which becomes greater with increasing expression of the POI, inhibitors or activators of the POI can be distinguished from agents that act upon other cell metabolites to effect a phenotypic change. Specification, p. 12, ll. 22-28. In preferred embodiments, the ratio of production by the "overproducing" cell line to production by the "native" line is maximized. See, e.g., Specification, p. 11, ll. 4-9; p. 53, Table 2. Such cells are sensitive in their growth properties to chemical agents that are capable of modulating the activity of the POI. See, e.g., Specification, p. 12, l. 30 to p. 13, l. 7. With regard to PKC, the specification shows that disclosed cell lines are highly sensitive and responsive both to agents which activate PKC as well as to those which inhibit PKC. Moreover, the Applicant provided the first evidence demonstrating that tamoxifen is capable of inhibiting the cellular functioning of the β1 isoform of Protein Kinase C (PKC). See Specification, p. 38, l. 26 to p. 39, l. 26.

In Applicant's own experience, the disclosed method is useful for identification of inhibitors or activators of a given POI. Although Applicant would not deny that there may be instances in which false positives arise, they are exceptional - the Applicant knows of none. By following the disclosed method, one of ordinary skill in the art can expect to identify substances that are inhibitors or activators of a protein-of-interest. In contrast, prior art screening methods can be used to identify large numbers of substances with biological activity, but it is problematic to determine which protein or other component of the cell such substances act on. There is no assurance that *any* of the substances exert their effect on the POI.

As indicated above, it is well within the ability of one of ordinary skill to confirm that a chemical agent identified by the method is indeed interacting with (*i.e.*, binding to) a given POI, if desired. The practitioner can rely on the specification (e.g., an

intact cell binding assay) or substitute any appropriate assay known in the art (e.g., a simple purified protein-drug binding assay).

Thus, Applicant asserts that every step necessary for successful practice of the claimed invention is disclosed, and respectfully requests that the rejection be withdrawn.

Rejection Under 35 U.S.C. § 112, second paragraph

Claims 33-34, 36-37, 43-50, 59-65, and 71-78 are rejected under 35 U.S.C. § 112 second paragraph as being indefinite with respect to the nature of control and test cell lines and genetic vectors.

A skilled practitioner would understand that the control cell line is not vastly different from the test cell, but should be as similar as possible to the test cell line to avoid difficulty in analyzing results, "preferably alike except for their expression (production) of the protein of interest at different levels (and any further differences necessitated by that difference in expression)." Specification, p. 8, ll. 26-29. It is understood that the cells should be sufficiently alike to show a responsive changes in a phenotypic characteristic of the test cell evoked by the production of the POI.

A skilled practitioner would also understand from the disclosure that it is not necessary to make or select a control cell that is otherwise identical to the test cell. Even where the test cell and control cell are not otherwise identical, the method is applicable, so long as a phenotypic characteristic affected by expression of the protein of interest is detectable. Inhibitors or activators are identified by their greater effect on the phenotype of the higher producing cell line. That this is the case is apparent, for example, from Tables 1(a-c), which present the results from overexpression of PKC in three different cell lines (Rat-6, NIH-3T3, C3H-10T1/2). The uninfected cell lines may serve as convenient controls for the PKC-overproducing cell lines.

Similarly, one of ordinary skill in the art would understand that a "second genetic vector" lacking a gene insert should otherwise be as similar as possible to a "first

genetic vector" containing a gene encoding a protein of interest. However, it would be understood that the difference in phenotypic characteristic relied upon for identification of inhibitors and activators should result from the differential expression of the protein of interest, but that other differences between the first and second vector were not excluded.

Applicant asserts that the meaning of the claims is clear. In order to identify activators or inhibitors of a POI, one of ordinary skill would understand to select a responsive change in a phenotypic characteristic evoked by the production of a POI in a cell, choosing cell lines or genetic vectors that are similar enough that a change in the responsive phenotypic characteristic is detectable. Depending on the phenotypic characteristic chosen, the skilled practitioner would know which differences in cell line or genetic vector were to be avoided.

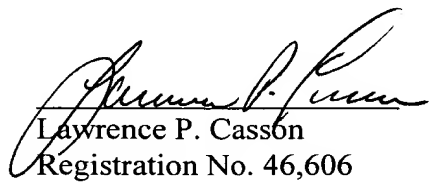
Accordingly, the claims are not indefinite and the rejection should be withdrawn.

It is believed that this amendment is fully responsive to the Examiner's rejection and allowance of the claims is respectfully requested.

Respectfully submitted,

KENYON & KENYON

Date: August 5, 2002

By: 

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